A High Throughput Zebrafish Embryo Gene Expression System for Screening Endocrine Disrupting Chemicals

Gloria V. Callard
Professor of Biology
Boston Univ., Boston MA 02215
(617) 353-8980
gvc@bu.edu

Key Words: chemicals, screening, genes, effects, exposure

Reproduction and development in man and animals are essential for survival of species, species diversity, maintenance of ecosystems, and commercial activities. Thus, there is an urgent need for regulators to develop methods to better predict which of the estimated 87,000 chemicals in the environment have the potential to disrupt hormone-dependent processes of development, physiology, and reproduction (EDC, endocrine disrupting chemicals). We propose development of an assay using living zebrafish (*Danio rerio*) embryos as a whole animal *in vitro* screening system for simultaneous detection of multiple subsets of EDC: (a) EDC that act via estrogen receptors (ER) to induce brain P450 aromatase (P450aromB) and hepatic vitellogenin (vtg) expression; (b) EDC that act via arylhydrocarbon receptors (AhR) to reduce gonadal aromatase (P450aromA) and indcrease P4501A1 expression; (c) EDC that interact directly with preformed aromatase enzyme to block aromatization; and (d) EDC that perturb ER and AhR expression *per se*. An automated real-time quantitative reverse transcription-polymerase chain reaction (qRT-PCR) approach will be used to measure targeted mRNAs in single and multiplex assays. The proposed zebrafish embryo system is a novel alternative to, and extension of, the current EDSP Tier 1 Screening Battery, which includes a mandate for ER binding and reporter assays and an alternative placental aromatase (enzyme) assay, but does not presently include an assay for chemicals that disrupt endogenous estrogen signaling by altering aromatase or ER expression, nor does it include an assay that can detect possible AhR mediated effects on reproductively relevant gene targets, or a screening assay that can simultaneously compare sensitivity and responsiveness of multiple genes to a given chemical. Although the proposed *in vitro* assay minimizes animal and chemical use, it has the advantages of an *in vivo* system for predicting agonist vs. antagonist properties of a chemical without *a priori* knowledge of uptake and accumulation, activating or metabolizing pathways, access to targets, receptor binding and activation, or required coregulators. Resultant data will provide biologically relevant criteria for prioritizing chemicals for further testing and will help to interpret reports of reproductive and developmental effects in wildlife and humans. Validation of a zebrafish embryo gene expression assay for detecting known and suspected ER- and AhR-acting EDC will have immediate applicability for routine chemical screening, and will demonstrate the feasibility of the same approach to detect chemicals that interact with other members of the nuclear receptor superfamily.